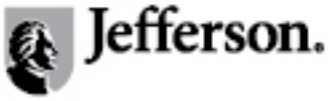

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Abstract

Endocrine signaling via nuclear receptors (NRs) is known to play an important role in normal physiology as well as in human tumor progression. Hormones regulate gene expression by altering local chromatin structure and, thereby, accessibility of transcriptional co-regulators to DNA. Recently it has been shown that non-histone proteins involved in hormone signaling, such as nuclear receptors and NR co-activators, are regulated by acetylation, resulting in their altered transcriptional activity. NAD-dependent protein deacetylases, the sirtuins (Sir2-related enzymes), directly modify NRs. Because sirtuins have been shown to regulate tumor cellular growth, aging, metabolic signaling and endocrine hormone signaling, they might play a role in cancer progression. This review focuses on the role of acetylation and the sirtuins in nuclear hormone receptor signaling.

Introduction

Histone modification

In eukaryotes, genomic DNA is packaged by histones and non-histone proteins into chromatin, which is further organized as repeating units of nucleosomes. The nucleosome consists of 147 base pairs of DNA wrapped around a protein core of histones (an H3–H4 tetramer and two H2A–H2b dimers) [5]. Histone proteins consist of a globular domain and a more flexible charged N-terminus, known as the histone tail. Covalent modifications of the histone tail include acetylation, phosphorylation, methylation and ubiquitination. Such modification of chromatin structure is evident during cell-cycle progression, DNA replication, DNA damage and repair, recombination and overall chromosome stability [5].In addition,

alteration to histones and non-histone proteins by acetylation plays a role in transcriptional activation and DNA-binding affinity, protein stability, protein–protein interactions, protein transport within the cell and nucleus, and activity of molecular chaperone proteins [6].

The acetylation status of histone proteins alters gene transcription. Removal of acetyl groups from lysine residues results in compaction of chromatin and, hence, repression of gene transcription [6]. As such, transcriptionally active euchromatin is typically hyperacetylated, compared with inactive heterochromatin which tends to be hypoacetylated [7].Inaddition, the N-terminal modification of histone tails provides recognition sites for factors involved in gene transcription. Acetylation of lysine residues provides a recognition motif for bromodomains. Other post-translational modifications of histones serve to recruit additional proteins with enzymatic activity and/or tethering functions for other proteins: methylation is recognized by chromo-like domains of the Royal family and phosphorylation is recognized by 14–3-3 proteins. Methylated histones (e.g. H3K4-Me3) recognize the PDH domain contained within BPTF, which recruits the NURF complex.

Post-translational modification of histones, in turn, can modify subsequent access of other enzymes. Distinct modifications of lysines are mutually exclusive. Binding of proteins might be excluded or enhanced by such modifications, and a catalytic activity of an enzyme can be modified by local enzymatic changes. For example, the phosphorylation of H3S10 regulates the binding of HP1 to methylated H3K9. Furthermore, isomerization of H3P38 governs methylation of H3K36 by Set 2. The prevailing evidence suggests that signal transduction pathways might coordinate sequential and carefully orchestrated post-translational modification of histones with distinct enzymatic activity. Although historically the notion of a histone code was used to describe the role of modifications in governing DNA functions, an expanded view considers the post-translational modification of histones to encode precise information governing gene expression. The hypothesis of the ‘histone code’ was initially proposed to be responsible for extending the information potential of genetic material within a cell [5].

Multiple proteins with acetyl transferase activity have been identified, and they are most commonly referred to as histone acetyl transferases (HATs). HATs are classified on the basis of their subcellular location: type A HATs are usually localized in the nucleus, whereas type B can be found in the cytoplasm. Type A HATs are involved in transcriptional regulation through their acetylation of nucleosomal histones within local chromatin. However, within the cytoplasm, type B HATs are thought to possess a ‘house-keeping’ role, acetylating newly formed free histones. Alterations in expression, translocation, amplification or mutationof genes encodingHATshave been found in several cancers [6]. One example is the breast-cancer susceptibilitygene BRCA2,which exhibits acetyltransferase activity [8].

The histone deacetylases (HDACs) have been divided into four groups based on their similarity to yeast transcriptional repressors. Class I and II HDACs are similar to the yeast Rpd3p and Hda1p proteins. Class III HDACs share similarity to the yeast transcriptional repressor Sir2p and are referred to as sirtuins. Class I and II HDACs are characterized by their sensitivity to inhibition by trichostatin A (TSA), whereas class III HDACs are nicotinamide adenine dinucleotide (NAD)-dependent. A fourth class includes the deacetylase HDAC11 [9]. This review will concentrate on the class III HDACs, the sirtuins.

Nuclear receptor modification by acetylation

Since the identification of p53 as a non-histone target of HATs, a growing body of evidence has shown an important role for acetylation and deacetylation of non-histone proteins in cellular physiology [10]. Several transcription factors, including the nuclear hormone receptors (estrogen receptor α (ERα), androgen receptor (AR), thyroid hormone receptor β (TRβ) and glucocorticoid receptor (GR)), p53 [11,12], GATA-1 [13], GATA-2 [14], GATA-3 [15], EKLF [16] and HMG proteins [17], are altered by acetylation. Acetylation of transcription factors results in altered binding to DNA, transcriptional activation, protein stability, subcellular localization and altered protein–protein interactions. For example, the tumor suppressor p53 is acetylated by p300, which functions as a rate-limiting cointegrator in diverse nuclear receptor signaling pathways, within its C-terminal domain, resulting in sequence-specific DNA binding [11] both *in vitro* and *in vivo* [12]. Recent studies have demonstrated that the individual acetylation sites of p53 determine its DNA binding [18]. Differential acetylation at distinct acetylation sites results in altered downstream effects, including cell survival after DNA damage, interaction with co-repressors and co-activators, interaction with downstream promoters, nuclear retention and DNA binding.

The AR plays a crucial role in prostate cancer proliferation as androgens induce proliferation and inhibit apoptosis. Androgen ablation therapy remains an important therapeutic in the management of metastatic prostate cancer. Hyperactivity of the AR is associated with loss of responsiveness to androgen therapy and androgen-insensitive prostate cancer (AIPC). Post-translational modification of the AR, including hyperacetylation, probably contributes to this hyperactivation. Acetylation of the AR by HATs (e.g. CBP and p300) regulates ligand binding and alters the affinity of the AR for co-regulators. Acetylation of the AR might thereby regulate prostate cancer growth and cell death [19].

The AR was the first nuclear receptor that was shown to be directly acetylated [20]. The residues of the AR that are acetylated *in vitro* by p300 are conserved among species and with other nuclear receptors [21]. Point mutation of the AR acetylation site to create acetylation mimic mutants enhanced ligand-dependent transactivation [20]. By contrast, acetylation dead mutants of the AR abrogated ligand-dependent transactivation. Although several functions are regulated by acetylation, including transactivation and contact-independent growth, the transrepression cAMP-and Akt-regulated function of the AR remained unaffected [22].

In vivo, acetylation of the AR is enhanced by agonists such as bombesin and its ligand dihydrotestosterone [23]. The physiological function of the acetylation site of the androgen receptor includes regulation of cellular growth [24].The introduction of AR acetylation mimic mutants into human prostate cancer cells promoted the growth of cells both *in vitro* and *in vivo*. This enhanced cellular growth was a function of both enhanced cellular proliferation and reduced cellular apoptosis. This finding was of importance because it was the first study to demonstrate that acetylation of a transcription factor directly regulated contact-independent growth. The molecular mechanisms and genetic targets regulating the cell survival and proliferation phenotype remain to be characterized completely.

It was previously shown that the AR can induce apoptosis. Substitution mutations of the AR acetylation sites abrogated tumor necrosis factor-α (TNF-α) and trail-induced apoptosis of human prostate cancer cells in culture. AR activity is induced by mitogen-activated protein kinase (MAPK) kinase kinase, which induces MKK4, Jun N-terminal kinase (JNK) and IκB kinases. MEKK1 induces prostate cancer cell apoptosis in an AR-dependent manner through JNK. Co-expression of MEKK1 and the wildtype AR in the presence of dihydrotestosterone

induced apoptosis. Mutation of the AR acetylation site abrogated MEKK1-induced apoptosis. Collectively, these studies demonstrate that MEKK1 (Jun-kinase kinase)-dependent cellular apoptosis involves the AR acetylation site. In addition, AR acetylation site mimic mutants were resistant to MEKK1-dependent apoptosis [25].

Prostate cancer cells transduced with acetylation mimic mutants of the AR showed enhanced cellular proliferation and induction of Ki67 [24]. These cells exhibited increased expression of proliferative cell-cycle markers including cyclin D1. Chromatin immunoprecipitation assays demonstrated enhanced recruitment of the gain-of-function AR acetylation site mutants to the cyclin D1 promoter [24]. Biochemical analysis of the molecular mechanisms by which the AR acetylation site regulates gene transcription indicated that lysines 630, 632 and 633 play a key role in the recruitment of the co-activator protein p300 and the disengagement of co-repressor complexes including NCoR, HDAC1 and Smad3 [25]. *In vitro* analysis demonstrated the importance of these lysine residues in serving as docking sites for p300 [25,26] (Figure1).

Signaling cascades in transcription factors

Studies of histones have demonstrated the interplay among post-translational modifications. For example prior modification of histones by phosphorylation regulates subsequent acetylation. The identification of post-translational modification of transcription factors raised the possibility that, like histones, one post-translational modification (i.e. phosphorylation) of transcription factors might regulate subsequent modification (i.e. acetylation or methylation). This hypothesis indicates that sequential post-translational modifications of transcription factors by phosphorylation, acetylation and ubiquitination might determine the signaling specificity of a transcription factor. The model in which these sequential post-translational modifications occur within transcription factors has been called signaling cascades in transcription factors (SCITs).

Consistent with this model, the AR is modified by both acetylation and phosphorylation, and AR acetylation affects phosphorylation-dependent signaling. MAPK pathways activate the AR, and the AR is phosphorylated by several distinct signaling cascades. In view of previous studies suggesting that H3 phosphorylation and acetylation might be linked events [27], we investigated the role of the AR acetylation site in signaling by the key kinases implicated in AR signaling, MAPK, Akt and protein kinase A (PKA). Mutation of the AR acetylation site did not affect MAPK signaling [22]. The AR acetylation site mutants were, however, defective in regulation by HDAC inhibitors, Akt signaling and cAMP signaling. cAMP signaling normally regulates recruitment of the AR to an androgen response element in chromatin immunoprecipitation assays; however, this recruitment was abolished by AR acetylation site mutation [22]. Furthermore, the AR normally forms three molecular weight forms dependent upon phosphorylation. Mutation of the AR acetylation site abrogated formation of the 114kDa phosphorylated form. The cAMP pathway, once activated, results in rapid dephosphorylation of the AR, in part through the induction of PKA-induced phosphatases. Collectively, this analysis of the inter-relationship between phosphorylation and acetylation of the AR has shown that AR acetylation regulates cAMP and Akt, but not MAPK-dependent,signaling of the AR [22](Figure2).These studies are consistent with prior analysis in which MAPK signaling to the ERa was unaffected by mutations in the ERa acetylation site [21].

Chromatin access of the nuclear receptors (NRs) is regulated by the acetylation of local

histones and histone demethylases. Point mutation of the six distinct AR phosphorylation sites led to the identification of a single site capable of regulating HDAC responsiveness [22]. Chromatin immunoprecipitation assays demonstrated enhanced AR recruitment in the context of local chromatin, upon addition of HDAC inhibitors, or through treatment with DHT. Acetylation dead AR mutants were defective in both ligand- and HDAC-dependent recruitment to the endogenous PSA promoter [22]. These studies suggest that the acetylation status of the nuclear receptor itself regulates access to local chromatin. The model in which local enzymatic activity of HDACs regulates AR occupancy is consistent with a growing body of evidence that multiple distinct nuclear receptor modifying enzymes co-occupy AR-binding sites in the context of local chromatin (Figure 3). For example, histone demethylases have been shown to co-occupy nuclear hormone receptor sites, performing a gatekeeper function of preventing the occupancy of unliganded nuclear receptors into the context of local chromatin [28]. Many studies have dissected the components of the AR co-repressor and co-activator complexes (reviewed in Ref. [29]).

In addition to the AR, it has been demonstrated that several other nuclear receptors are acetylated. The ERa was initially shown to be acetylated by p300 at the same motif as the AR [21]. Mutational analysis identified a minimal region sufficient for acetylation and an ERα peptide encoding the site served as substrate for p300 HAT activity with similar properties to H3. Proteomic analysis demonstrated that Lys302 and Lys303 are preferential sites for acetylation. Independent clinical study identified Lys K303 as a site of frequent mutation in breast cancer [30]. Like p53, in which multiple distinct acetylation sites have been identified [18], additional acetylated ERα Lys residues were identified recently (K266/2681) in assays combining SRC1 with p300.

The regulation of glucocorticoid receptor activity by acetylation is complex. HDAC6 regulates HSP90-dependent GR maturation [31]. The GR is acetylated after ligand binding. Mutagenesis of the acetylated residues (K494/ 495) reduced GR acetylation and GR-mediated repression of nuclear factor-kB (NFkB) activity [32]. The addition of thyroxine results in the accumulation of acetylated TRb within 30 to 45 min. Inhibition of MAPK activity reduced thyroxine-induced TRb acetylation [33].

In addition to the nuclear receptors and histones, several other components of the nuclear receptor signaling cascades are substrates for acetylation. For example, kinases (MEK2 and IKKa) and nuclear receptor co-regulators (p300, MTA1, PGC1a ACTR, and HDACs) also serve as substrates for acetylation by HATs. Collectively, these studies indicate an important link between the acetylation of non-histone proteins and nuclear hormone receptor signaling (Figure 3).

Sirtuins: Sirt1 and the nuclear sirtuins

The sirtuin family is a group of nicotinamide (NAD^+)-dependent deacetylases that are conserved from archaeobacteria to eukaryotes [4,34]. The sirtuins were originally classified as type III HDACs, because histones were the first identified substrates of the yeast Sir2p gene [35]. Sir2 conveys NAD^+-dependent ADP-ribosyltransferase activity, that is, the enzymatic activity of sirtuins is regulated by NAD^+. In mammals, there are seven homologues of the yeast Sir2 gene, SIRT1–SIRT7, each with significant diver sity in function (Table 1).

Mammalian sirtuins can be localized to the nucleus, cytoplasm or mitochondria, with distinct substrates and a broad range of functions [2]. The structure of the sirtuins includes a conserved core domain, with all seven homologues differing in their N-or C- terminal sequences [35]. SIRT1 is the human homolog of the yeast silent information regulator 2 (Sir2) gene. Endogenous levels of nicotinamide probably limit Sirt1 activity, while the mechanisms regulating Sir2 activity might be regulated by either altered ratios of cellular NAD^+:NADH [36] or free nicotinamide, and free NAD^+ levels might suppress Sir2 activity [37].

The three nuclear sirtuins (SIRT1, SIRT6, and SIRT7) have distinct functions. SIRT1 is known to deacetylate more than ten substrates, including the nuclear receptors (AR, ERα), the NR co-activators (PGC1α, p300) [38–40], Ku70 and several transcription factors that are regulated by p300 (NFκB, FKHR). SIRT6 has weak deacetylase activity but significant auto-ADP-ribosyltransferase activity. In an outbred background, $SIRT6^{-/-}$ mice develop defective skeletal fusion, osteoporosis, low insulin-like growth factor1 (IGF-1) levels, and hypoglycemia. Genomic instability in these mice is associated with enhanced sensitivity to DNA damaging agents and defective base excision repair. SIRT7 exhibits a nucleolar distribution within proliferating, but not quiescent, cells. This sirtuin lacks NAD^+-dependent deacetylase activity and interacts with RNA polymerase I. An NAD^+-binding motif in SIRT7 indicates that this family member might function to sense NAD^+ concentrations in proliferating cells and thereby regulates RNA Pol I activity.

Cytosolic SIRT2 binds and deacetylates α-tubulin and histones, while preferentially deacetylating H4K16 [41]. Expression of SIRT2 is reduced in some cancers [42] and is increased during mitosis co-localizing with chromatin during the G2M phase [43]. SIRT2 deacetylation of cytoskeletal proteins might regulate migration and/or their intracellular cargo in response to altered intracellular metabolism.

The extended sirtuin family

The mitochondrial sirtuins (SIRT3, SIRT4 and SIRT5) appear to regulate mitochondrial respiratory function. Both SIRT3 and SIRT4 are imported into the mitochondrial matrix [44,45]. SIRT3 is required for expression of the uncoupling protein 1 (UCP-1) [46]. SIRT3 deacetylates and activates acetyl-coenzyme-A synthetase (AceSC2) [44, 47], thereby facilitating the entry of acetate into the tricarboxylic acid (TCA) cycle. Polymorphisms of SIRT3 have been linked to longevity [48,49]. SIRT4 ADP-ribosylates mitochondrial glutamate dehydrogenase, and subsequently inhibits its activity and blocks the conversion of glutamate to α -ketoglutarate. The cellular interactions and biological significance of SIRT5 are not yet known, although its structure suggests a deacetylase function.

Sirtuins and nuclear receptors

In addition to the TSA-sensitive histone deacetylases, NAD-dependent histone deacetylases (the sirtuins) regulate histone and non-histone protein structure and function and are important for nuclear hormone receptor signaling. One non-histone protein that is deacetylated by a sirtuin, Sirt1, is the co-activator p300 [39]. Sirt1 represses p300 in a NAD^+-dependent manner. The p300 HAT contains multiple distinct domains, including the cell cycle regulatory domain (CRD1), and lysine residues within the CRD1 domain are required for Sirt1

repression of p300. The Lys residues within p300 function as substrates for Sirt1-mediated deacetylation, as well as for sumoylation. Additionally, the SUMO-specific protease, SSP3, antagonizes Sirt1-mediated repression of p300 [39]. Collectively, these studies demonstrate a key role for Sirt1 in regulating p300 deacetylation. Sirt1 regulation of p300 might thereby integrate metabolic signals within the cell, because diverse nuclear receptors are regulated by p300.

In view of the key role for the AR acetylation site in regulating prostate tumor cellular growth *in vivo*, together with evidence that the incidence of prostate cancer increases with aging and the finding that the sirtuins might play a role in organismal aging, the potential role of the sirtuins in regulating AR function has been investigated. The AR has been shown to be repressed by SIRT1 in prostate epithelial cells. Consistent with this, confocal microscopy revealed co-localization of the AR and SIRT1 in a nuclear subcompartment [38]. SIRT1 inhibited cellular proliferation in AR-expressing prostate cancer cell lines but not in cells that do not express AR [38], demonstrating that their interaction is physiologically relevant. Inhibition of SIRT1 with antagonists increased androgen-regulated gene transcription. Importantly, inhibitors of endogenous Sirt1 (Sirtinol, splitomycin and nicotinamide) induced endogenous AR gene expression. The repression of AR activity by Sirt1 required the catalytic function of Sirt1. The AR lysine residues that are acetylatedbyp300 serve as substrates for Sirt1-mediated deacetylation (Figure3). The ability of SIRT1 to deacetylate the AR and repress its activity might provide a novel and effective cancer therapy.

What might be the physiological significance of the finding that Sirt1 inhibits AR activity? This model is consistent with a known role of androgens in maintaining muscle mass and in the induction of genes associated with muscle development. The AR is known to play a key role in several biological functions. In addition to maintaining muscle mass and reducing fat mass, the AR governs the pathological state driving tumors and the progression of prostate cancer. The metabolic changes induced by pyruvate increase the NAD^+:NADH ratio in muscle cells thereby inhibiting muscle gene expression. Conversely the induction of lactate, which would reduce NAD^+, might stimulate muscle gene expression [50].

In the case of prostate cancer, recent studies have demonstrated that global changes in histone acetylation occur, in particular acetylation of H3K18, H4K12 and H3K9 [51]. These changes in acetylation are thought to be predictive of prostate cancer recurrence [51]. The role of Sirt1 in altering histone acetylation during prostate cancer progression and/or through AR function is currently unknown. However, it is known that during prostate cancer progression, cells shift towards cytosolic glycolysis [52]. The induction of lactate that occurs during prostate cancer progression [53] might be anticipated to inhibit Sirt1 and thereby enhance AR function [38].In prostate cancer cells, Sirtinol, a noncompetitive inhibitor of Sirt1, induced expression of the endogenous AR gene, as did the selective chemical Sirt1 inhibitor, splitomycin [38]. Therefore, it is proposed that Sirt1 might inhibit prostate cancer cellular growth by inhibiting AR function.

Subsequent studies have demonstrated that other nuclear receptors, including the ERα, TRβ and GR, contain a functional conserved acetylation motif. The role of sirtuins in regulating these receptors is not known at this time; however, there is indirect evidence for the regulation of ERα by sirtuins. The ERα is acetylated by p300 at lysine residues within its hinge and ligand-binding domains [54]. These lysine residues are well conserved between

species and with other nuclear receptors. Resveratrol was thought to function as a specific SIRT1 activator [55] and is an agonist for the ER [56]. However, resveratrol has diverse biological effects [57], including antioxidant properties, inhibition of mitochondrial ATPases and competition with co-enzyme Q, and this has led to the substrate specificity of resveratrol being called into question. Resveratrol was shown to enhance binding and deacetylation of peptide substrates that contain fluorophore (Fluor de Lys), a non-physiological fluorescent moeity, but not a similar acetylated peptide without a fluorophore. Resveratrol did not exert detectable effects on Sir2 activity (transcriptional silencing near telomeres, rDNA silencing) [58,59].

Resveratrol has chemopreventative properties [60], such as inhibiting preneoplastic mammary gland lesions [60]. The role of resveratrol in breast cancer cellular proliferation remains controversial [61,62].Inthe estrogen-dependent breast cancer cell line T47D, resveratrol promoted cell proliferation [56], whereas Mgbonyebi et al. [63] showed an anti-proliferative effect of resveratrol in both ER-positive and ER-negative breast cancer cells. It has been reported that resveratrol has estrogenic and anti-estrogenic properties. A prevailing view is that resveratrol is a selective estrogen receptor modulator (SERM) that functions as an ER agonist in some tissues and as an ER antagonist in others. Further clarificationis necessary to determine whether resveratrol functions through SIRT to regulate ERα activity and whether this agent will have a clinical benefit in women diagnosed with breast cancer.

Sirtuins and metabolism

The sirtuin-dependent regulation of nuclear receptor coactivators has been linked to the regulation of glucose metabolism. Calorie restriction promotes insulin sensitivity and subsequent reductions in blood glucose and insulin levels [64]. Recent studies have linked Sirt1 to the nuclear receptor co-regulator PGC1a. SIRT1 regulates hepatic gluconeogenesis by promoting increased gluconeogenesis and by repressing glycolytic gene function. Through deacetylation of PGC1-a, SIRT1 enhances gluconeogenic gene transcription via interactions with hepatocyte nuclear factor 4a [40] associated with repression of glycolytic genes. SIRT1 regulation of FOXO1 activity in hepatocytes has also been shown to enhance gluconeogenesis and glucose release [65].In addition, SIRT1 regulates insulin secretion in pancreatic b-cells [66,67]. These two groups have demonstrated that this mechanism is dependent on SIRT1-mediated repression of the uncoupling protein 2(UCP2) gene.

A growing body of evidence has suggested a role for estrogen and estradiol-dependent gene transcription in regulating double-stranded DNA break repair [68].In this regard addition of estradiol induces transient double-stranded DNA breaks to which procyclic acidic repetitive protein (PARP) is recruited. Recent findings suggest a role for sirtuins in double-stranded DNA break repair. SIRT6 is predominantly a nuclear protein, with high levels of expression in mouse muscle, brain and heart [69]. Recent evidence points to a role for SIRT6 in resistance to DNA damage and suppression of genomic instability [70]. $SIRT6^{-/-}$ mice die within four weeks of birth after developing abnormalities that include loss of subcutaneous fat, lordokyphosis and severe metabolic defects [70]. Furthermore, MEFs deficient in SIRT6 show impaired proliferation and increased sensitivity to DNA damaging agents [70]. Haigis and Guarente [2] propose that SIRT6 can ADP-ribosylate substrate proteins involved in the base excision repair pathway, a mechanism by which SIRT6 could be regulating DNA damage repair [70].

At this time the role of nuclear receptors in aging and their relationship to the function of sirtuins in aging is unknown. Restriction of calorie intake extends lifespan in several organisms, including yeast, flies, spiders, worms and rodents [2]. On the basis of the observation that Sir2 extension of lifespan in *Caenorhabditis elegans* involves the forkhead protein, Dauer formation 16 (DAF-16), a model has been proposed in which caloric restriction prolongs life through Sir2 signaling in conjunction with the DAF-2 insulin-like signaling pathway. SIRT1 expression is induced by calorie restriction and fasting, suggesting a role for SIRT1 in the regulation of fasting responses. Increased dosages of Sir2 homologues are also capable of increasing lifespan [71]. It has been proposed that Sir2 promotes longevity in yeast cells by reducing recombination of rDNA (rDNA) and the subsequent formation of extrachromosomal rDNA circles (ERCs). Strains deficient in SIR2 and NPT1 do not show an extension in lifespan [72], thereby emphasizing the importance of NAD^+-activated Sir2p in prolonging life.

Conclusion

At least eight distinct types of post-translational modification of histones have been described. Several of the enzymes responsible for these modifications directly modify nuclear receptors. Acetylation, like phosphorylation, is now known to govern cellular growth [24]. The addition of ligand to nuclear receptors induces receptor acetylation [23]. Nuclear receptors contain intra-receptor signaling modules consisting of cascades of phosphorylation, acetylation, ubiquitination and perhaps sumoylation [73]. Histone-modifying enzymes, including histone acetylases and arginine methyltransferases, co-associate with most nuclear receptors in the context of local chromatin [28]. The finding that sirtuins directly regulate nuclear hormone receptors provides a new mechanism by which nuclear receptors might coordinate metabolic signaling. Local metabolic changes in NAD^+;NADH ratios sensed by the sirtuins might, in turn, regulate local activity of nuclear receptors. As nuclear receptors are located in distinct subcellular compartments, including the cellular membrane, mitochondria, cytoplasm and nucleus, and distinct sirtuins occupy discrete subcellular compartments, it will be of interest to determine the role of the sirtuins on nuclear receptor activity in these distinct subcellular locales. The finding that SIRT1 regulates the nuclear receptor co-integrator p300 provides a new mechanism by which local NAD^+:NADH changes might coordinate nuclear receptor function within the cell.

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Figures and Tables

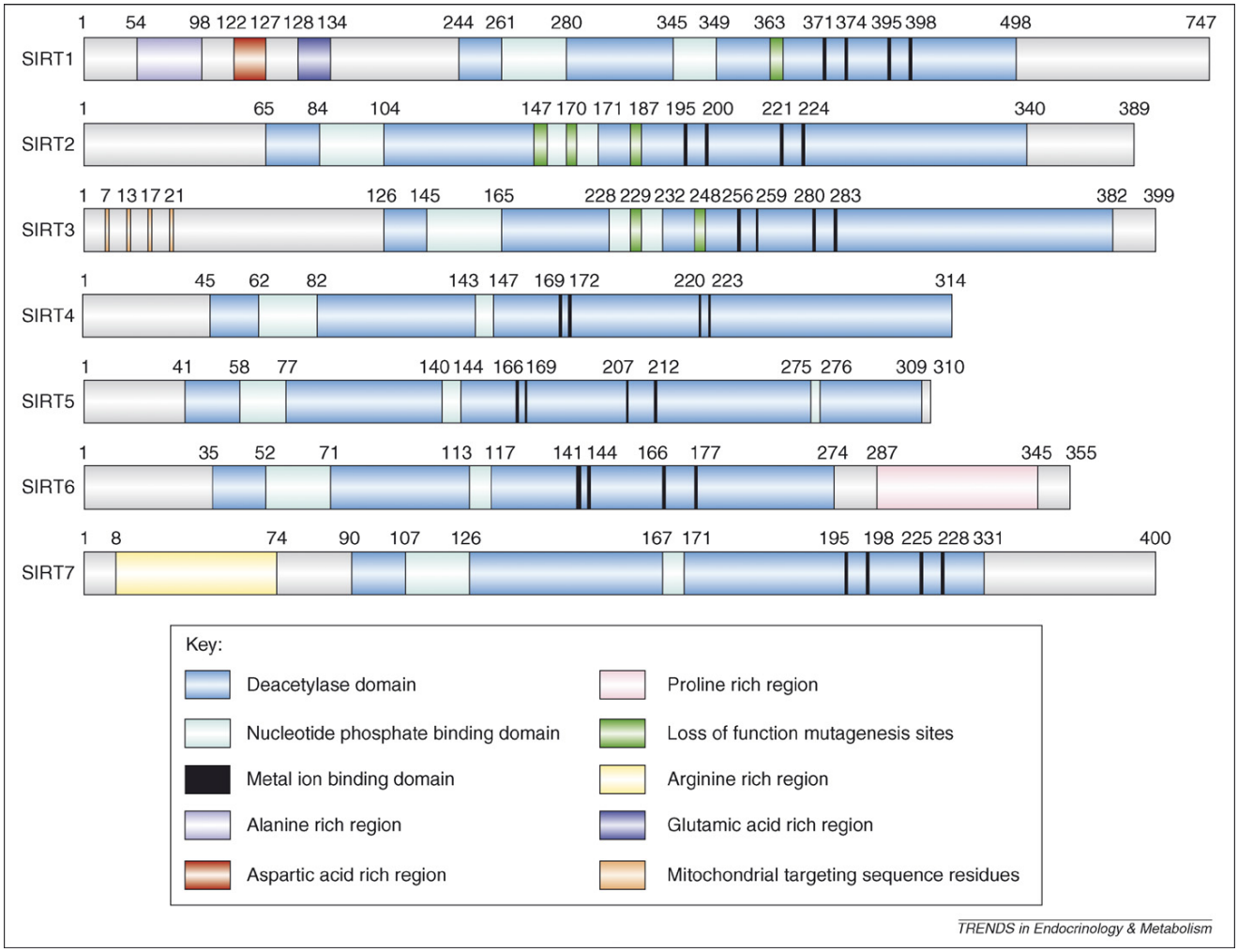

Figure 1

Domain architecture of human sirtuin family of Class III NAD^+-dependent histone deacetylases Schematics represent the domain structure of human sirtuins. Amino acid positions are noted above each schematic. The domains are represented in different colors. SIRT1 is not drawn to the same scale as the other sirtuins. Adapted from UniProt Universal Protein Resource Database.

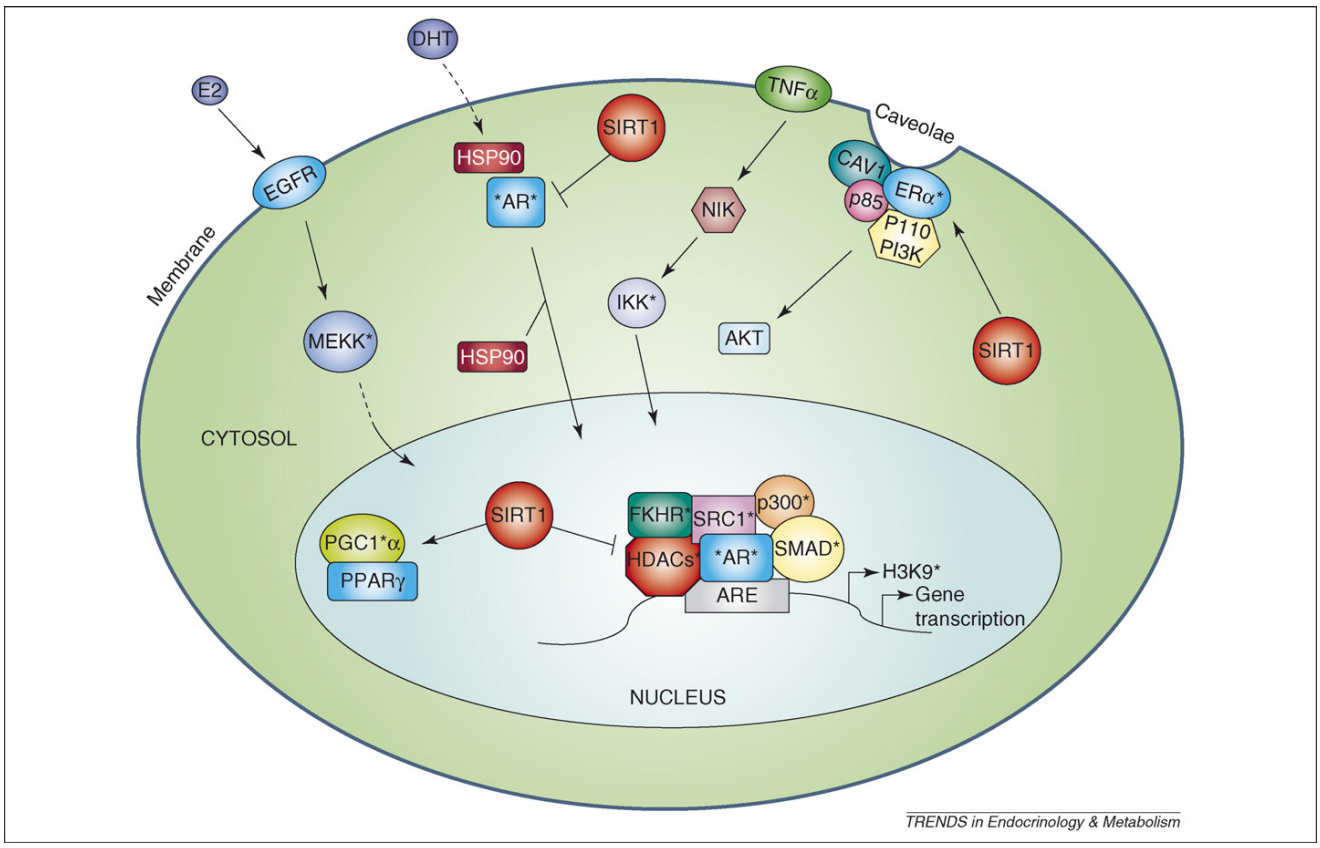

Figure 2

Proteins involved in ligand-induced hormone signaling are acetylated nuclear receptor ligands that induce multiple downstream molecular interactions. Proteins known to be involved in nuclear receptor signaling are shown. Proteins with an asterisk have been shown to serve as substrates for acetylation in vitro both within the cytosol and in the nucleus.

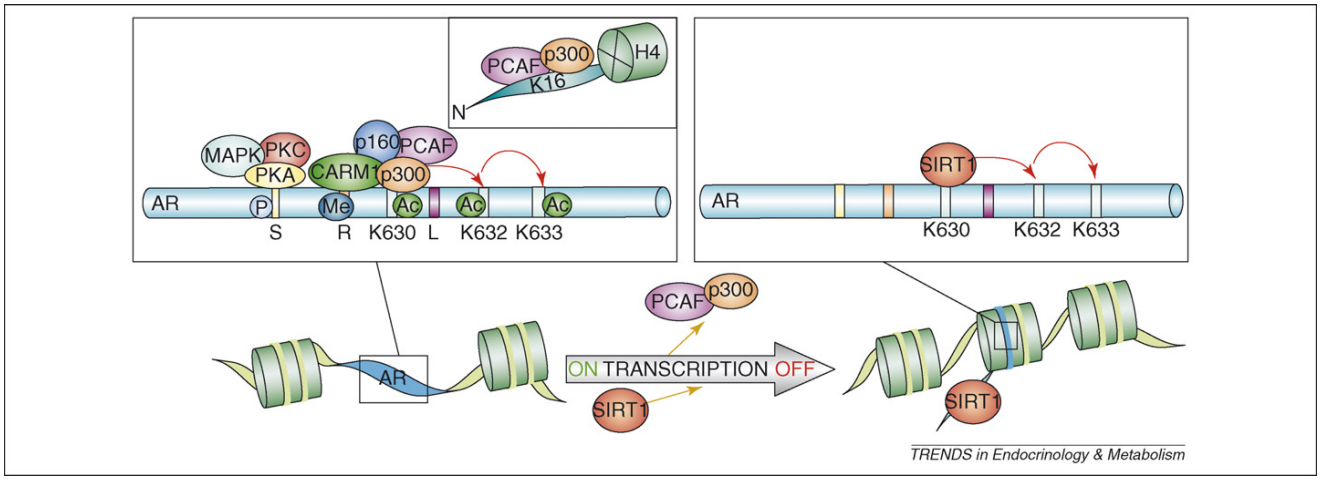

Figure 3

Transcriptional regulation of the androgen receptor through epigenetic modifications. Acetylation and subsequent activation of the androgen receptor is mediated by p300 and P/CAF at the indicated Lys residues. The AR is also capable of being methylated by CARM1 and phosphorylated by MAPK, PKC or PKA at multiple serine residues. The exact location of such residues remains a controversial area of research. Because these modifications are mutually exclusive, but can occur on the same residues, the possibility of 'crosstalk' between modifications is suggested. Deacetylation and, therefore, transcriptional repression is elicited by SIRT1. Also shown within the figure insert is the interaction between histone acetyltransferases P/CAF and p300 as they associate with H4K16.

Table 1. The functions, interactions and subcellular location of the mammalian sirtuins[a]

Sirtuin	Function	Chromosomal location	Subcellular localization	Physiological location	Known interactions	Biological implications
SIRT1	Deacetylase	10q21.3	Nucleus (33) PML bodies (49) Cytoplasm (cell-type specific) (50)	Liver, Muscle Pancreas, Testis, Ovary, Adipose tissue	FOXO,PGC-1α, AR, ER, p53, Ku70, NFkB, HES1	Cell survival DNA damage repair Metabolism Lipid and glucose homeostasis Stress resistance Insulin secretion Axonal degradation (50)
SIRT2	Deacetylase	19q13.2	Cytosol (33)	Heart, Brain, Testes, Skeletal muscle	α-Tubulin, H4, HOXA10	Cell cycle control Intracellular transport Cell motility
SIRT3	Deacetylase	11p15.5	Mitochondria (33)	Heart, Brain, Testes, Liver, Kidney, Muscle, Brown adipose tissue	AceCS2, UCP-1, PGC-1a	Thermogenesis Metabolism
SIRT4	ADP-ribosyltransferase	12q24.31	Mitochondria (33)	Expressed in all tissues except leukocytes in adults and thymus in the fetus (49)	GDH	Insulin secretion Metabolism
SIRT5	Deacetylase	6p23	Mitochondria (33)	Broadly expressed in adult and fetal tissues	Unknown	Unknown
SIRT6	ADP-ribosyltransferase	19p13.3	Nucleus (29,33)	Unknown	DNA Polβ	DNA repair (36) Glucose homeostasis (50)
SIRT7	Unknown	17q25.3	Nucleolus (33)	Spleen, Ovary, Thyroid, Liver, Tissues with dividing cells (50)	RNA Pol I	rDNA transcription

[a]Adapted from Refs [35,74].

Table 1

The functions, interactions and subcellular location of the mammalian sirtuins[a]

[a] Adapted from Refs [35, 74].